GENERAL METHOD FOR THE SYNTHESIS OF ATP GAMMA-DERIVATIVES

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1. Introduction

Nucleoside triphosphate analogues modified in the terminal phosphate are active in reactions catalyzed by NTP utilizing enzymes [1–5]. For example, ATP gamma-anilidate inhibits the aminoacylation of tRNAs [1–3], and serves as a substrate for RNA-polymerase [4]. Several ATP gamma-analogues are used for affinity labeling [2,3]. Available methods for the preparation of these compounds involve multi-stage procedures providing low or moderate yields [5–8].

The present communication describes a single-stage method for quantitative conversion of ATP to various gamma-derivatives based on (1) the acid-catalyzed activation of ATP with DCC resulting in the accumulation of adenosine-5′-trimetaphosphate (I, fig.1.) [9,10], and (2) the reactions of I with strong bases of a wide range of nucleophilic reactivity in solutions of reduced acidity. Procedures are presented for the synthesis of several ATP gamma-analogues including one bearing an alkylating 2-chloro-ethylamino group [11].

Abbreviations: DCC- dicyclohexyl carbodiimide, DMSO-dimethyl-sulfoxide, DMF-dimethylformamide, TEA-triethyl-amine, TLC–thin-layer chromatography, IEC–ion-exchange chromatography. Roman numerals. (I,II a–f) refer to adenosine-5′-trimetaphosphate and the ATP gamma derivatives displayed in fig.1.

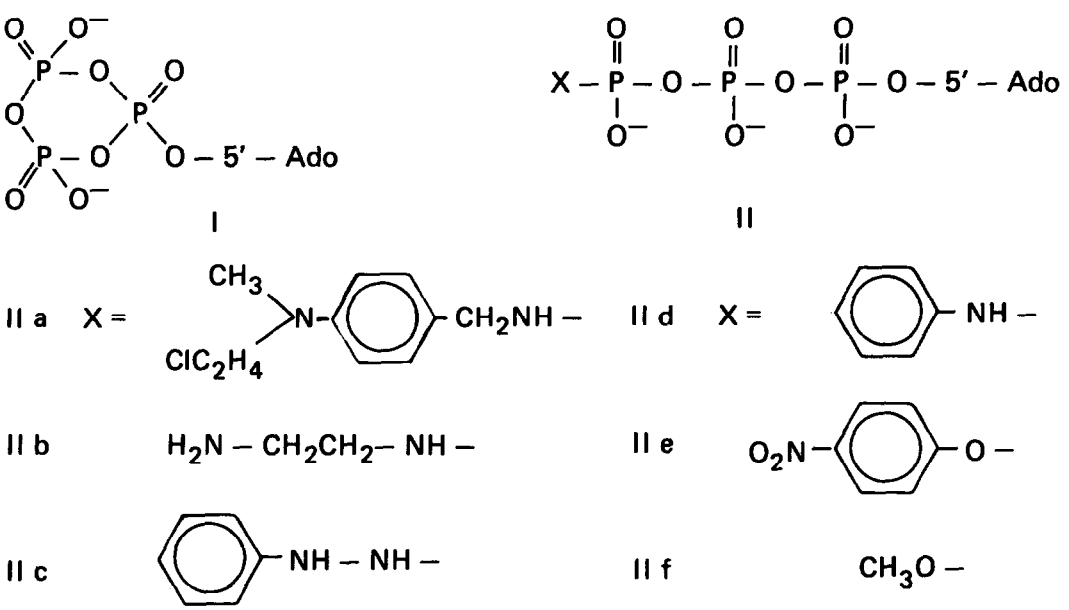

Fig.1

2. Experimental

ATP (95–97% pure) was from Reanal. 4-(*N*-2-chloroethyl-*N*-methylamino)-benzylamine dihydrochloride was prepared as described in [12]. Other chemicals were of reagent grade.

[^{31}P]NMR spectra were taken with a Brucker HX-90 pulse spectrometer operating at 36.43 MHz. The external standard was 85% H_3PO_4. Chemical shifts are in ppm.

The purity of preparations was checked by (1) TLC on Merck silica gel plates F_{254} in the solvent system iso-butyric acid–conc. NH_3–water (66:1:33) and (2) IEC in 7 M urea using on MSFP-3 spectrophotometer manufactured at this Institute. Chlorine in alkylating reagents was determined after hydrolysis with 1 M KOH at 100°C for 1 h by potentiometric titration of the resulting Cl^- with silver nitrate.

2.1. *Activation of ATP*

ATP (0.15 mmol dry triethylammonium salt) was dissolved in 1.5 ml of either DMSO or DMF-methanol mixture (9:1) and supplemented with 0.5 mmol DCC. The mixture was dried by evaporation with 0.1 ml pyridine. After 1–2 h [^{31}P]NMR analysis revealed 100%. I, 24 ppm. An aliquot of the reaction mixture (0.02 ml) was treated with 1 M ethylene diamine (0.2 ml, pH 8.0), diluted to 10 ml and chromatographed on DEAE cellulose column. Found: IIb (fig.1), 97%; ADP, 3%. It is important that the solvents be dry. If the humidity content is above 0.005%, a greater excess of DCC must be taken to obtain I in a quantitative yield.

IIa

4-(*N*-2-chloroethyl-*N*-methylamino)-benzylamine dihydrochloride (0.5 mmol), was dissolved in 2 ml of cold methanol saturated with NH_3. Ammonium chloride was precipitated with ether, and the clear solution was evaporated. The resulting oil was taken up in methanol (0.1 ml). 0.05 ml TEA was added to ensure the complete deprotonation of the amine. 0.1 mmol I was treated by this mixture in 1 ml of DMF for 5 h at 5–10°C. IIa was precipitated with ether, washed twice and dissolved in methanol. It was converted to sodium salt by treatment with 1 M sodium acetate in methanol (1 ml). Found: IIa 99–100% (IEC). ATP 1.03 μmol/mg, after the hydrolysis by 0.05 M HCl at 40°C for 1 h. Cl^- 0.00 before and 0.98 μmol/mg after hydrolysis with alkali. Yield, 80 mg.

IIb

0.1 mmol of I in DMF was added to 1 M neutral solution of ethylene diamine in water. Found: IIb 100% (^{31}P-NMR). The solution was dried by evaporation with pyridine. IIb was obtained as the sodium salt as described for IIa. Yield, 55 mg.

IIc

0.06 mmol of I in 0.5 ml of DMF were treated with 3 mmol of freshly distilled phenyl hydrazine in the presence of thiophenol (0.02 ml). Readily oxidizable product IIc was isolated in the presence of 2-mercaptoethanol. Found: IIc 100%. Yield, 50 mg.

IId

I (0.07 mmol) was treated by 0.9 ml of aniline in 0.8 ml of DMF at 25–30°C for 70 h. Found: IId, 98.6%; ADP, 1.4%. Yield, 78 mg.

IIe

I (0.08 mmol in 0.9 ml of DMF) was treated by a mixture of 4-nitrophenol and triethylamine (1.5 mmol each) for 24 h. Found: IIe 100% (IEC). Yield, 60 mg.

IIf

ATP (0.06 mmol) was treated with DCC (150 mg) in 1 ml of DMF–methanol mixture (1:1 v/v). The mixture was supplemented with TEA (0.2 ml) and kept at 35–40°C for 3 days. Found: IIf, 92%. The admixture is inorganic trimetaphosphate (23 ppm), as suggested by the ^{31}P-NMR spectrum of the reaction mixture [10]. Yield, 25 mg.

3. Results and discussion

No simple method for quantitative conversion of ATP to various gamma-analogues is available in the literature. Several gamma-amidates were prepared by the activation of ATP with DCC in the presence of the amine that has to be phosphorylated [6,7]. Since the activation is an acid-catalyzed process, this method does not provide good yields with basic amines.

It seems reasonable to activate ATP with DCC and to phosphorylate bases with the activated product I in media differing in acidity. The advantage of this approach has been taken for the preparation of several ATP gamma-amidates in the preceeding work [11].

Treatment of ATP with DCC results in a rapid and quantitative formation of I. The two doublets corresponding to alpha and gamma phosphorus atoms in the ^{31}P-NMR spectrum of the reaction mixture disappear within 1 h, and the only signal observed is a multiplet centered at 24 ppm [10,11]. As it is evidenced by ^{31}P-NMR spectroscopy, the product is remarkably stable in DMSO or in DMF and may be stored in the absence of moisture for days, while it is unstable in water or in the strongly nucleophilic solvent, pyridine [10,13]. The extent of activation of ATP may also be determined using the reaction of I with ethylene diamine. The product IIb may be separated from ATP by any chromatographic technique.

The addition of bases to I affords exclusively the corresponding ATP gamma-derivatives (II fig.1.), usually in a quantitative yield. It is convenient to follow this process by ^{31}P-NMR technique. The characteristic changes in the position and in the multiplicity of the resonance signal of gamma phosphorus atom suggest that the substitution takes place at the gamma position.

ATP gamma-esters are available by this procedure if one adds TEA, a base catalyst. The latter seems to have been omitted from the reaction mixture by earlier workers [14] who attempted to obtain IIe in essentially the same way.

Properties of ATP gamma-derivatives are summarized in table 1.

The conditions of the synthesis of IIa are mild enough to provide the stability of the 2-chloroethylamino group [15], so that IIa is contaminated with less than 1% alkylation products.

The above described operations with I and nucleophilic compounds are simple and reproducible. The scope of this method was recently extended to the preparation of oligonucleotide 5′-triphosphate gamma-amides [16]. It seems likely that this procedure should be regarded as a rather general method of gamma-substitution in triphosphate derivatives.

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Table 1
Properties of ATP gamma-derivatives

	Chemical shifts of phosphorus atoms					UV-spectra, pH 7	
	alpha	beta	gamma	solvent	R_f (TLC)	240/260	290/260
ATP	12.7	23.6	11.2	DMSO	0.18	0.35	0.01
	11.7	23.9	10.9	DMSO-methanol 1:1			
IIa	11.6	21.4	2.2[a]	DMSO-methanol 1:1		0.48	0.04
IIb	11.2	21.2	1.8[a]	DMSO-water 1:1		0.35	0.01
IIc	11.4	22.1	4.2	pyridine	0.45	0.83	0.31
IId					0.47	0.85	
IIe	14.0	24.5	19.7	DMF-pyridine 1:1	0.50	0.41	0.47
IIf	12.7	24.0	11.5[b]	DMF-methanol 1:1	0.30	0.35	0.01

[a] Doublet of triplets
[b] Doublet of quadruplets

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